Journal of Helminthology

cambridge.org/jhl

Research Paper

Cite this article: Costa NA, Simões RO, Vilela RV, Souza JGR, Cardoso ST, Leiner NO, Gentile R, Maldonado AJunior (2018). Morphological and genetic characterization of *Pterygodermatites* (*Paucipectines*) *zygodontomis* (Nematoda: Rictulariidae) from *Necromys lasiurus* (Rodentia: Sigmodontinae) from Uberlândia, Brazil. *Journal of Helminthology* **92**, 618–629. https://doi.org/10.1017/S0022149X17000736

Received: 27 March 2017
Accepted: 21 July 2017
First published online: 4 October 2017

Author for correspondence:
A. Maldonado, E-mail: maldonad@ioc.fiocruz.br

CAMBRIDGE UNIVERSITY PRESS

Morphological and genetic characterization of *Pterygodermatites* (*Paucipectines*) *zygodontomis* (Nematoda: Rictulariidae) from *Necromys lasiurus* (Rodentia: Sigmodontinae) from Uberlândia, Brazil

N.A. Costa[1,2], R.O. Simões[1,2], R.V. Vilela[2], J.G.R. Souza[2], S.T. Cardoso[3,4], N.O. Leiner[4], R. Gentile[2] and A. Maldonado Junior[2]

[1]Programa de Pós-Graduação em Biodiversidade e Saúde, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, Av. Brasil 4365, Rio de Janeiro, RJ, 21040-360, Brasil; [2]Laboratório de Biologia e Parasitologia de Mamíferos Silvestres Reservatórios, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, Av. Brasil 4365, Rio de Janeiro, RJ, 21040-360, Brasil; [3]Programa de Pós-Graduação em Ecologia e Conservação dos Recursos Naturais, Instituto de Biologia, Universidade Federal de Uberlândia, Rua Ceará s/n, Uberlândia, MG, 38405-315, Brasil and [4]Laboratório de Ecologia de Mamíferos, Instituto de Biologia, Universidade Federal de Uberlândia, Rua Ceará s/n, Uberlândia, MG, 38405-315, Brasil

Abstract

Pterygodermatites (Paucipectines) zygodontomis, a nematode parasite of the small intestine of the rodent *Necromys lasiurus*, from Uberlândia, Minas Gerais state, Brazil, was analysed by light and scanning electron microscopy. Additionally, phylogenies were inferred from the mitochondrially encoded cytochrome *c* oxidase I gene (MT-CO1). Details of the helminth surface, such as the oral aperture, cephalic papillae, papillae in the posterior region of the body and longitudinal cuticular elements represented by spine-like projections and fans are presented, adding new taxonomic details. Molecular phylogenetic analysis, based on the MT-CO1, demonstrated that *P.* (*P.*) *zygodontomis* and *Pterygodermatites (Paucipectines) jaegerskioldi* form a unique evolutionary unit in accordance with the subgenus *Paucipectines* and corroborated their occurrence in cricetid and didelphid hosts.

Introduction

Parasitism is one of the most common interactions among species within ecosystems (Gardner & Campbell, 1992; Thomas *et al.*, 2005), and it affects individual health, population growth, community structure and even ecosystem functioning (Grenfell, 1992; Morand *et al.*, 1996; Morand & Arias-Gonzalez, 1997; Poulin, 1998). Small mammal hosts, including rodents, marsupials and bats, are infected by a variety of parasitic species, and may act as reservoirs of zoonosis, such as leishmaniasis, Chagas disease and schistosomiasis (Gentile *et al.*, 2010; Orozco *et al.*, 2014). Despite the importance of studies concerning mammal–parasite interactions for addressing the occurrence of diseases in natural and human-disturbed environments (Simões *et al.*, 2010; Moreira *et al.*, 2013; Cardoso *et al.*, 2016), little is known about the helminth fauna, their geographic distribution, host specificity and prevalence in Neotropical small mammals. Moreover, studies containing detailed taxonomical and structural descriptions of these helminths are still scarce.

Necromys lasiurus (Lund, 1840) is a small (35 g), terrestrial rodent species which is known to be a reservoir of leishmaniasis (Brandão-Filho *et al.*, 2003) and hantavirus pulmonary syndrome (Limongi *et al.*, 2013). Its distribution extends through central Brazil to south of the Amazon River, including north-eastern Argentina, extreme south-eastern Peru, Paraguay and Bolivia (Redford & Eisenberg, 1999), thus inhabiting the grasslands of Cerrado, Caatinga, as well as open areas in the Atlantic Forest biome in Brazil (Bonvicino *et al.*, 2008). It is considered to be a generalist species, being favoured by anthropogenic disturbances due to its tolerance of habitat modification and broad diet spectrum, including leaves, seeds, fruits and insects (Vieira & Baumgarten, 1995; Redford & Eisenberg, 1999). Moreover, due to its high density and short life cycle (Francisco *et al.*, 1995), *N. lasiurus* may contribute to persistence of parasite populations and their transmission among hosts (Oliveira *et al.*, 2014; Sabino-Santos *et al.*, 2016).

Nematodes of the family Rictulariidae Froelich, 1802, infect mammals worldwide, including bats, marsupials, rodents, carnivores and primates. The morphological characteristics of the genus *Pterygodermatites* are based on the oral opening position and the total number cuticular projections, the number of prevulvar cuticular projections in females, and the positions of the

papillae at the posterior end and the size of the spicules in males (Quentin, 1967). *Pterygodermatites* (*Paucipectines*) *zygodontomis* was first described by Quentin (1967) from the small intestine of the rodent *N. lasiurus* collected in Exú, in the state of Pernambuco, Brazil. However, some morphological aspects were not described in detail, and this species has not been reported by scanning electron microscopy and molecular analysis.

Within the phylum Nematoda, phylogenetic studies based on molecular data have been carried out for the order Spirurida by Blaxter *et al.* (1998), Wijová *et al.* (2005) and Nadler *et al.* (2007). Nevertheless, so far, no molecular phylogeny has included the family Rictulariidae. In fact, just one small sequence from a member of this family is available in public databases.

In the present study, *P. (P.) zygodontomis* was analysed by light and scanning electron microscopy, adding new details to taxonomic characteristics. Additionally, phylogenies including representatives of this nematode genus were inferred, based on partial sequences of the mitochondrially encoded cytochrome *c* oxidase I gene, to determine relationships of the superfamily Rictularioidea within the order Spirurida.

Materials and methods

Study sites and habitat description

Rodents were captured in Uberlândia (18°55′07″S, 48°17′19″W), in the state of Minas Gerais, Brazil, which is located in the Cerrado biome. Cerrado is the largest South American savannah, characterized by a tropical climate with a dry winter from April to September and a wet season from October to March. Collection was carried out in rural areas, including grassland and cornfields, and in the borders of Cerrado vegetation preserved areas, called Cerrado *sensu stricto*.

Collection and examination of rodents

In collaboration with the staff of the municipal government, 102 specimens of *N. lasiurus* were collected and analysed for helminth parasites during an investigation of hantavirus cases. Animals were captured with Tomahawk® (16 × 5 × 5 inches) and Sherman® traps (3 × 3.75 × 12 inches) baited with a mixture of peanut butter, banana, oats and bacon. Trapping occurred between December 2011 and November 2012. Biosafety techniques were used during all procedures involving biological samples (Lemos & D'Andrea, 2014).

Helminth recovery and morphological analysis

The abdominal and thoracic cavities of the rodents were examined for the presence of helminths. Organs were placed separately in Petri dishes, washed twice in physiological saline solution, and dissected under a stereomicroscope. Worms were washed twice in saline solution to remove tissue debris and fixed in AFA (2% glacial acetic acid, 3% formaldehyde, 95% ethanol), or alternatively preserved in 70% ethanol for DNA isolation. For study of morphological characters, nine male and ten female specimens were cleared in 80% phenol (70% ethanol and phenolic acid), mounted on temporary slides and examined using a Zeiss Scope Z1 light microscope (Zeiss, Göttingen, Germany). The structures were measured via digital images captured by a Zeiss Axio Cam HRC using the accessory software Axio Vision Rel. 4.7.

For scanning electron microscopy (SEM), six specimens (three males and three females) were fixed in 2.5% glutaraldehyde and 4% freshly prepared formaldehyde in 0.1 M cacodylate buffer, pH 7.2, washed in 0.1 M cacodylate buffer, post fixed for 2 h in 1% osmium tetroxide and 0.8% potassium ferricyanide, pH 7.2, dehydrated in a graded ethanol series (20–100° GL) for 20 min each step and dried to critical point (Mafra & Lanfredi 1998). Specimens were examined using a JEOL JSM-6390 microscope (JEOL, Tokyo, Japan) at the Rudolf Barth Electron Microscopy Platform of the Oswaldo Cruz Institute.

Voucher specimens of the helminths were deposited in the Coleção Helmintológica do Instituto Oswaldo Cruz (*P. zygodontomis* numbers: CHIOC 38398, female only, from Exú, Pernambuco state and CHIOC 38399, male and female, from Uberlândia, Minas Gerais state; *Pterygodermatites jaegerskioldi* numbers: CHIOC 38400 and 38501 from Corumbá, Mato Grosso do Sul state).

Molecular and phylogenetic analysis

Genomic DNA samples were isolated from mid-section fragments of *P. zygodontomis*, *P. jaegerskioldi*, *Physocephalus lassancei* and *Protospirura numidica* adult worms (table 1). DNA isolation used the QIAGEN QIAamp® DNA Mini Kit according to the manufacturer's protocol (QIAGEN, Hilden, Germany). Each reaction was performed using only one specimen. Before DNA extraction, each specimen was clarified in alcohol/glycerol, identified morphologically and subsequently washed in 70% ethanol and distilled water.

DNA amplification by polymerase chain reaction (PCR) methodology was conducted using the primer cocktail: NemF1_t1 5′-TGT AAA ACG ACG GCC AGT CRA CWG TWA ATC AYA ARA ATA TTG G-3′, NemF2_t1 5′-TGT AAA ACG ACG GCC AGT ARA GAT CTA ATC ATA AAG ATA TYG G-3′, NemF3_t1 5′-TGT AAA ACG ACG GCC AGT ARA GTT CTA ATC ATA ARG ATA TTG G-3′, NemR1_t1 5′-CAG GAA ACA GCT ATG ACT AAA CTT CWG GRT GAC CAA AAA ATC A-3′, NemR2_t1 5′-CAG GAA ACA GCT ATG ACT AWA CYT CWG GRT GMC CAA AAA AYC A-3′ and NemR3_t1 5′-CAG GAA ACA GCT ATG ACT AAA CCT CWG GAT GAC CAA AAA ATC A-3′, as described by Prosser *et al.* (2013), for the barcode region of the mitochondrial cytochrome *c* oxidase subunit I gene (MT-CO1). Each PCR contained 2.5 µl of 10× PCR buffer, 2 µl of 50 mM $MgCl_2$, 0.5 µl of each primer cocktail (10 µM of a three-forward-primers mix, and 10 µM of a three-reverse-primers mix), 0.5 µl of 10 mM deoxynucleotide triphosphate solution (dNTPs), 0.2 µl of Invitrogen™ Platinum™ *Taq* DNA polymerase (500 U/µl) (Invitrogen, São Paulo, Brazil), 2.0 µl of genomic DNA and ultrapure water, in a total reaction volume of 25 µl. Thermal cycling conditions were 94°C for 1 min; five cycles at 94°C for 40 s, 45°C for 40 s, 72°C for 1 min; followed by 35 cycles at 94°C for 40 s, 51°C for 40 s, 72°C for 1 min; and a final extension at 72°C for 5 min (Prosser *et al.*, 2013). The resulting amplicons were visualized on 2% agarose gels using GelRed™ nucleic acid gel stains (Biotium, Hayward, California, USA).

Successfully amplified amplicons were purified using the GE Healthcare illustra™ GFX™ PCR DNA and Gel Band Purification Kit following the manufacturer's protocol (GE Healthcare Little Chalfont, Bucks, UK) and then cycle sequenced using the Applied Biosystems™ BigDye™ Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Carlsbad, California, USA), individually for each primer mentioned above for better accuracy. Sequencing was performed using the Applied Biosystems™ ABI

https://doi.org/10.1017/S0022149X17000736 Published online by Cambridge University Press

Table 1. Species, geographic locality, host and GenBank accession number of species used for this study.

Species	Geographic locality	Host	Accession number	Reference
Acuarioidea				
Acuaria europaea	Bulgaria	*Dendrocopos syriacus*	KX353874	Mutafchiev *et al.*, 2017
Proyseria petterae	Madagascar	*Corythornis vintsioides*	KJ995862	Mutafchiev *et al.*, 2014
Camallanoidea				
Procamallanus spiculogubernaculus	India	*Heteropneustes fossilis*	KU292358	Chaudhary *et al.*, 2017
Dracunculoidea				
Dracunculus medinensis	n/a	n/a	JN555591	n/a
Philometroides sanguineus	China	*Carassius carassius*	KM111526	Su *et al.*, 2016
Filarioidea				
Acanthocheilonema viteae	n/a	*Meriones unguiculatus*	HQ186249	McNulty *et al.*, 2012
Brugia malayi	n/a	n/a	AF538716	Ghedin *et al.*, 2007
Brugia pahangi	n/a	n/a	AP017680	n/a
Brugia timori	n/a	n/a	AP017686	n/a
Chandlerella quiscali	United States	*Quiscalus quiscula*	HM773029	McNulty *et al.*, 2012
Litomosoides sigmodontis	n/a	n/a	AP017689	n/a
Loa loa	Cameroon	*Homo sapiens*	HQ186250	McNulty *et al.*, 2012
Onchocerca flexuosa	n/a	*Cervus elaphus*	HQ214004	McNulty *et al.*, 2012
Onchocerca ochengi	n/a	n/a	KX181290	n/a
Onchocerca volvulus	Brazil	*Homo sapiens*	KT599912	Crainey *et al.*, 2016
Setaria digitata	China	*Bubalus bubalis*	KY284626	Liu *et al.*, 2017
Wuchereria bancrofti	India	*Homo sapiens*	JN367461	Ramesh *et al.*, 2012
Gnathostomatoidea				
Gnathostoma doloresi	Japan	*Sus scrofa*	KX231806	Sun *et al.*, 2016
Gnathostoma spinigerum	China	*Monopterus albus*	KP410547	Liu *et al.*, 2015b
Gnathostoma nipponicum	Japan	*Mustela nivalis*	KX826911	Sun *et al.*, 2017
Habronematoidea				
Habronema muscae	Italy	*Equus caballus*	FJ471583	Iorio *et al.*, 2009
Habronema microstoma	Italy	*Equus caballus*	FJ471582	Iorio *et al.*, 2009
Physalopteroidea				
Physalopteridae gen sp. 1	Mexico	*Sceloporus* sp.	KC130693	Prosser *et al.*, 2013
Physalopteridae gen sp. 2	Mexico	*Imantodes* sp.	KC130708	Prosser *et al.*, 2013
Physaloptera sp. 1	Mexico	*Trimorphodon biscutatus*	KC130707	Prosser *et al.*, 2013
Physaloptera sp. 2	Mexico	*Trimorphodon biscutatus*	KC130706	Prosser *et al.*, 2013
Turgida sp.	Mexico	*Didelphis virginiana*	KC130680	Prosser *et al.*, 2013
Rictularioidea				
Pterygodermatites jaegerskioldi 1	Brazil	*Gracilinanus agilis*	KT894802	Present study
Pterygodermatites jaegerskioldi 2	Brazil	*Gracilinanus agilis*	MF155935	Present study
Pterygodermatites zygodontomis 1	Brazil	*Necromys lasiurus*	MF187069	Present study
Pterygodermatites zygodontomis 2	Brazil	*Rhipidomys mastacalis*	MF155934	Present study
Pterygodermatites zygodontomis 3	Brazil	*Necromys lasiurus*	MF155933	Present study
Spiruroidea				
Cylicospirura felineus	United States	*Lynx rufus*	GQ342967	Ferguson *et al.*, 2011
Cylicospirura petrowi	n/a	n/a	KF719952	n/a

(*Continued*)

https://doi.org/10.1017/S0022149X17000736 Published online by Cambridge University Press

Table 1. (*Continued.*)

Species	Geographic locality	Host	Accession number	Reference
Cylicospirura subaequalis	United States	*Puma concolor*	GQ342968	Ferguson *et al.*, 2011
Gongylonema aegypti	Egypt	*Acomys dimidiatus*	LC026046	Setsuda *et al.*, 2016
Gongylonema pulchrum	China	*Capra aegagrus hircus*	KM264298	Liu *et al.*, 2015a
Physocephalus lassancei	Brazil	*Thrichomys fosteri*	KT894799	Present study
Protospirura muricola	Central African Republic	*Gorilla gorilla*	KP760207	Lefoulon *et al.*, 2015
Protospirura numidica 1	Brazil	*Oxymycterus dasythricthus*	KT894800	Present study
Protospirura numidica 2	Brazil	*Oxymycterus delator*	KT894801	Present study
Spirocerca lupi	China	*Canis lupus familiaris*	KC305876	Liu *et al.*, 2013b
Thelazioidea				
Thelazia lacrymalis	Italy	*Equus caballus*	AJ271619	Casiraghi *et al.*, 2001
Thelazia callipaeda	China	*Canis lupus familiaris*	JX069968	Liu *et al.*, 2013a
Thelazia callipaeda	n/a	n/a	AP017700	n/a
Plectoidea				
Plectus aquatilis	Belgium	Free-living aquatic	KX017524	Kim *et al.*, 2017

n/a, Not available.

3730 DNA Analyzer. Both procedures and cycle-sequenced product precipitation were conducted at the DNA sequencing platform of the Oswaldo Cruz Institute, PDTIS/FIOCRUZ. Fragments were assembled into contigs and edited for ambiguities using the Geneious 9.1.8 software (http://www.geneious.com; Kearse *et al.*, 2012), resulting in a consensus sequence for each individual worm.

The MT-CO1 dataset included sequences from representatives of the superfamilies Rictularioidea (our two *Pterygodermatites* species), Acuarioidea, Camallanoidea, Dracunculoidea, Filarioidea, Gnathostomatoidea, Habronematoidea, Physalopteroidea, Spiruroidea and Thelazioidea (table 1). Plectoidea was included as the outgroup. Substitution saturation in the dataset was assessed using the test by Xia *et al.* (2003) and Xia & Lemey (2009) with the software DAMBE version 6.4.79 (Xia, 2017).

Phylogenetic reconstructions were carried out using the software Treefinder version of March 2011 (Jobb, 2011) for maximum likelihood (ML) as optimality criteria and MrBayes version 3.2.6 (Ronquist *et al.*, 2012) for Bayesian inference (BI). Both ML and BI analyses were performed using distinct models per codon position, to account for different evolutionary processes at each of the three codon positions.

In the ML analyses, evolutionary models were chosen by the Bayesian information criterion (BIC) (Schwarz, 1978). ML-pairwise distances were computed using the same codon-based partitioned models using Treefinder. Robustness of nodes in ML was assessed by non-parametric bootstrap percentages (BP) after 1000 pseudo-replicates and by the expected-likelihood weights applied to local rearrangements of tree topology (LR-ELW) after 1000 replicates.

In the BI analyses, distinct GTR + I + G models were used for each codon position, with unlinking of base frequencies and parameters. Markov chain Monte Carlo (MCMC) sampling was performed for 10,000,000 generations with four simultaneous chains, in two runs. Robustness of nodes in BI was assessed by Bayesian posterior probabilities (BPP) calculated from trees that were sampled every 100 generations, after removing the first 25% generations as a 'burn-in' stage. Adequacy of BI analyses sampling was assessed through the effective sample size (ESS) of each parameter, calculated using the software Tracer version 1.6 (Rambaut, 2012). Values above 100 effectively independent samples were considered sufficient.

Results

Morphology by light and scanning electron microscopy

Description

Adult helminths exhibited sexual dimorphism. The female body was larger and more robust than the male, and both showed two columns of spine-like cuticular projections organized in pairs located ventrally, beginning below the buccal capsule and extending until near the end of the body. The oral cavity was triangulate, surrounded by six labial papillae (2 ventral, 2 lateroventral and 2 dorsal) and four external cephalic papillae (1 pair ventral and 1 pair dorsal) (figs 1A and 2B–D). There was a pair of amphids between the lateral and the dorsal labial papillae (fig. 2B). The buccal capsule with thick walls was sclerotized, trapezoidal in shape, with three oesophageal teeth (fig. 1A). The oral opening was surrounded by a toothed strip of 21 denticles in females (9 dorsal and 6 on each ventrolateral side) and 17 in males (7 dorsal and 5 on each ventrolateral side) (figs 1A and 2E). The female presented 81 ventrolateral spine-like cuticular projections arranged in pairs, being 38 prevulvar and 43 postvulvar (fig. 1B). The vulvar opening was posterior to the oesophageal–intestinal junction (fig. 1C). The tail was conical and fig. 1D indicates the position of the anus.

Males presented 41 spine-like ventrolateral cuticular projections (fig. 2A) with three or four fans ventral and anterior to the cloaca in juvenile and adult worms, respectively, in finfold shape (figs 1E, 2A). The posterior end of the male had nine pairs of papillae: 2 pairs precloacal, 1 pair ad-cloacal and 6 pairs postcloacal, organized in two groups. The first group was made up of two pairs of papillae and the second with three pairs of papillae near the tail tip. The ninth pair of papillae was located at the tip of the male tail, ending bluntly (figs 3B–D). A pair of phasmids was also observed between the eighth and the

https://doi.org/10.1017/S0022149X17000736 Published online by Cambridge University Press

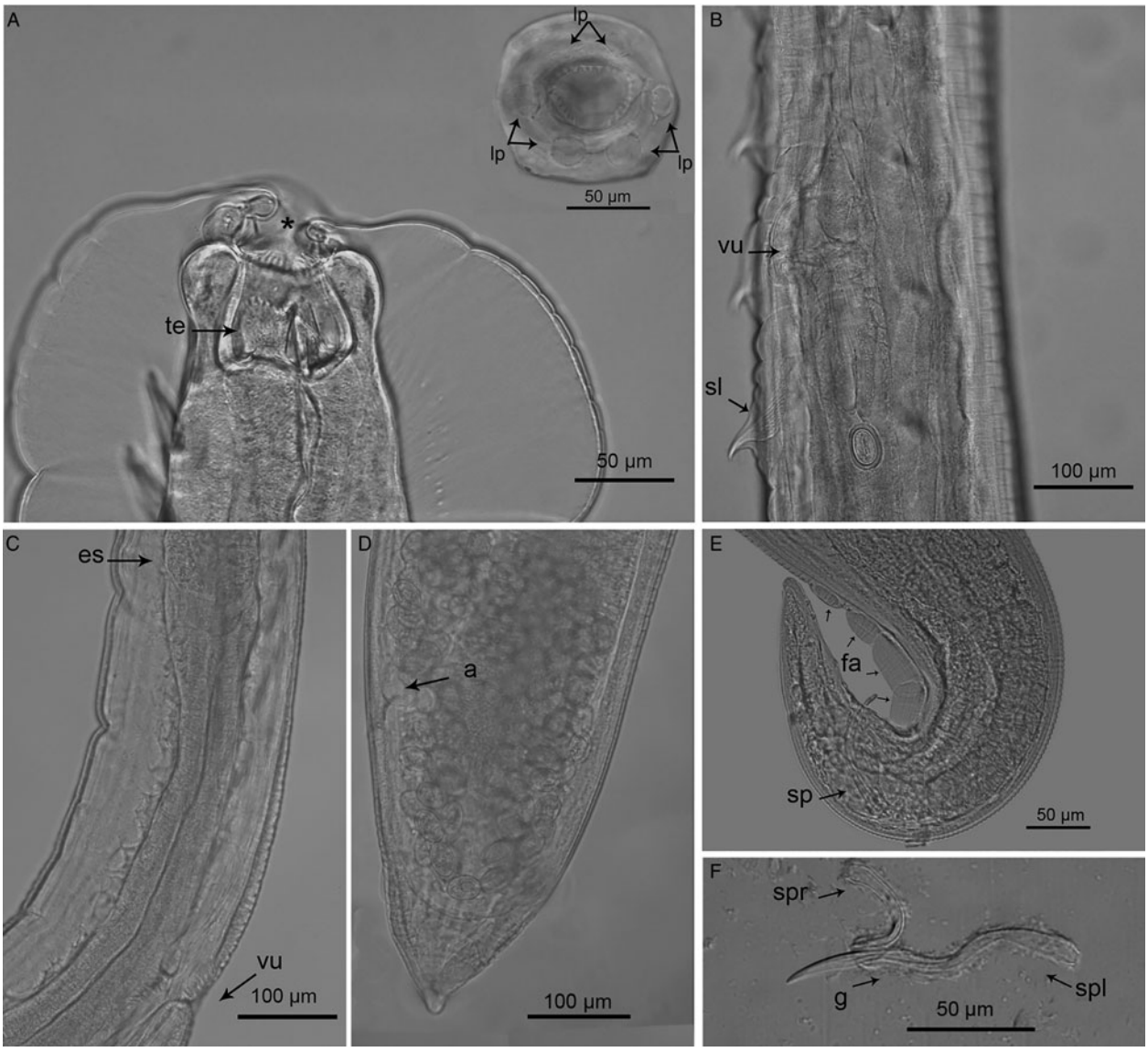

Fig. 1. Light microscopy of *Pterygodermatites* (*Paucipectines*) *zygodontomis*. (A) Female buccal capsule with three oesophageal teeth (te) showing oral opening (asterisk). Details of apical view showing three developed lips and six labial papillae (lp); (B) vulvar (vu) region, lateral view, and spine-like posvulvar cuticular projections (sl); (C) region of transition of oesophagus (es) and intestine and vulva (vu); (D) female posterior region, lateral view, anus (a); (E) male posterior region, ventral view, details of four fans (fa) and spicules (sp); (F) right spicule (spr), left spicule (spl) and gubernaculum (g), lateral view.

ninth pair of papillae (fig. 3C). The spicules were unequal in size with similar shape, at a ratio of 1:2 (right:left) (fig. 1F). The presence of a rectangular gubernaculum was observed (fig. 1F). These traits and the morphometric characteristics allowed us to identify the specimens to the species level by comparing them with other specimens belonging to *P.* (*P.*) *zygodontomis* recovered from *N. lasiurus* (table 2).

Molecular and phylogenetic analysis

Alignment of sequences resulted in a matrix comprising 46 taxa and 876 characters, of which 309 were constant and 507 were variable characters, informative for parsimony. The test by Xia & Lemey (2009) for substitution saturation provided evidence for saturation only at the third codon positions, whereas overall there was little saturation in the matrix (data not shown).

Our phylogenies, based on the MT-CO1, inferred using two different optimality criteria (ML and BI), resulted in similar topologies with little variation in nodes and support values (fig. 4). The ML method resulted in a tree with score lnL = –12339.81. The evolutionary models selected through BIC were: TN+G in the first codon positions, TVM+G in the second codon positions, and J2+G in the third codon positions, all models with optimized substitution rates, frequencies of bases and gamma distributions. The BI Markov chains provided highly significant estimated sample sizes (ESS) for all parameters.

MT-CO1 sequences formed two well-supported reciprocally monophyletic groups with *P. jaergerskioldi* (LR-ELW = 94%, ML-BP = 98%, BPP = 100%) and *P. zygodontomis* (LR-ELW = 89%, ML-BP = 98%, BPP = 100%). These two clades formed a strongly supported monophyletic group (LR-ELW = 100%, ML-BP = 100%, BPP = 100%), representing the superfamily Rictularioidea.

https://doi.org/10.1017/S0022149X17000736 Published online by Cambridge University Press

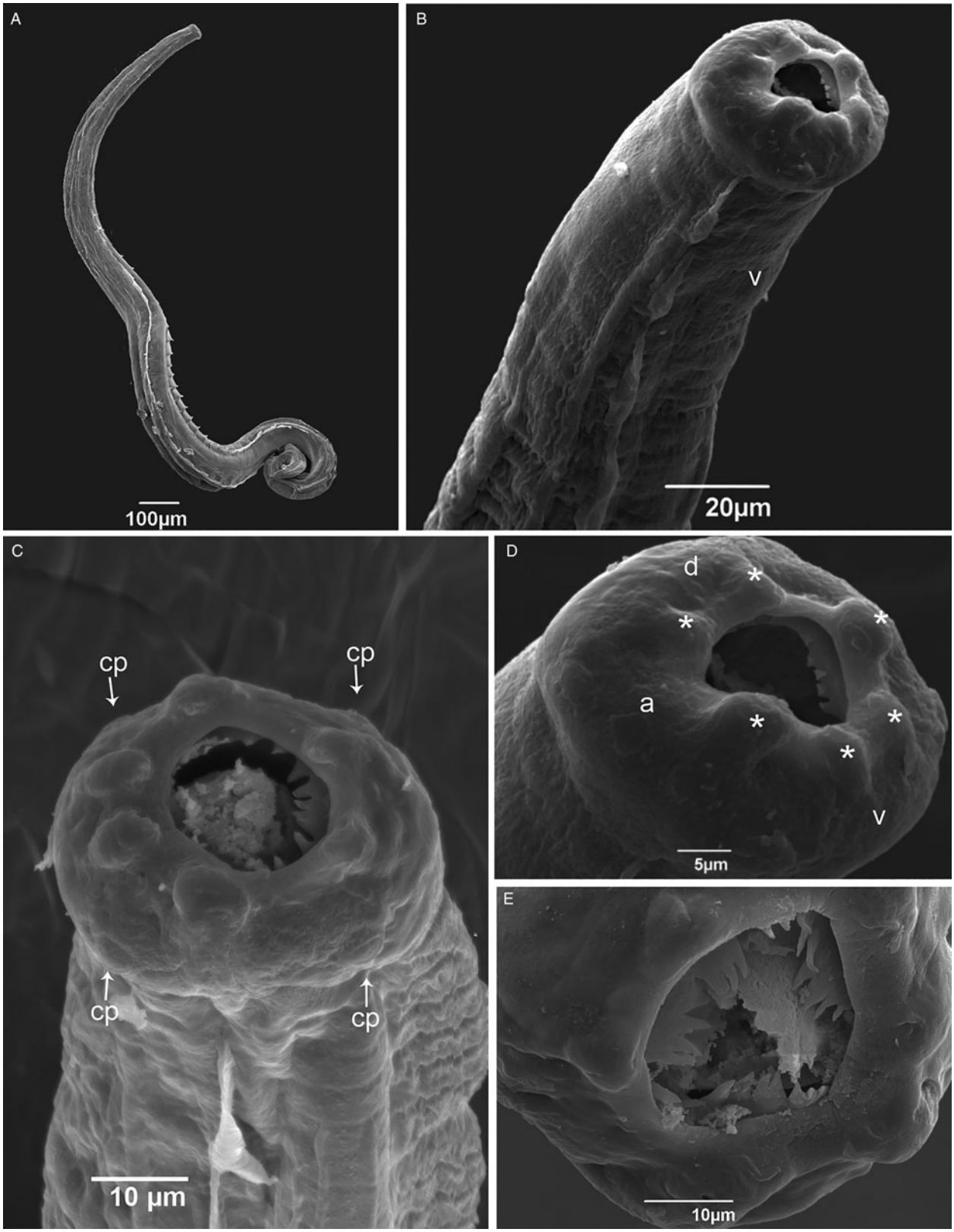

Fig. 2. Scanning electron microscopy of *P. (P.) zygodontomis*. (A) Male, spine-like cuticular projections; (B) female, anterior end showing a pair of well-defined longitudinal cuticular elements, ventrolaterally located (v); (C) female, anterior end, apical view and external cephalic papillae (cp); (D) apical view, amphid (a), ventral region (v), dorsal region (d) and labial papillae (asterisk); (E) anterior end showing oral opening surrounded by a crown of teeth.

https://doi.org/10.1017/S0022149X17000736 Published online by Cambridge University Press

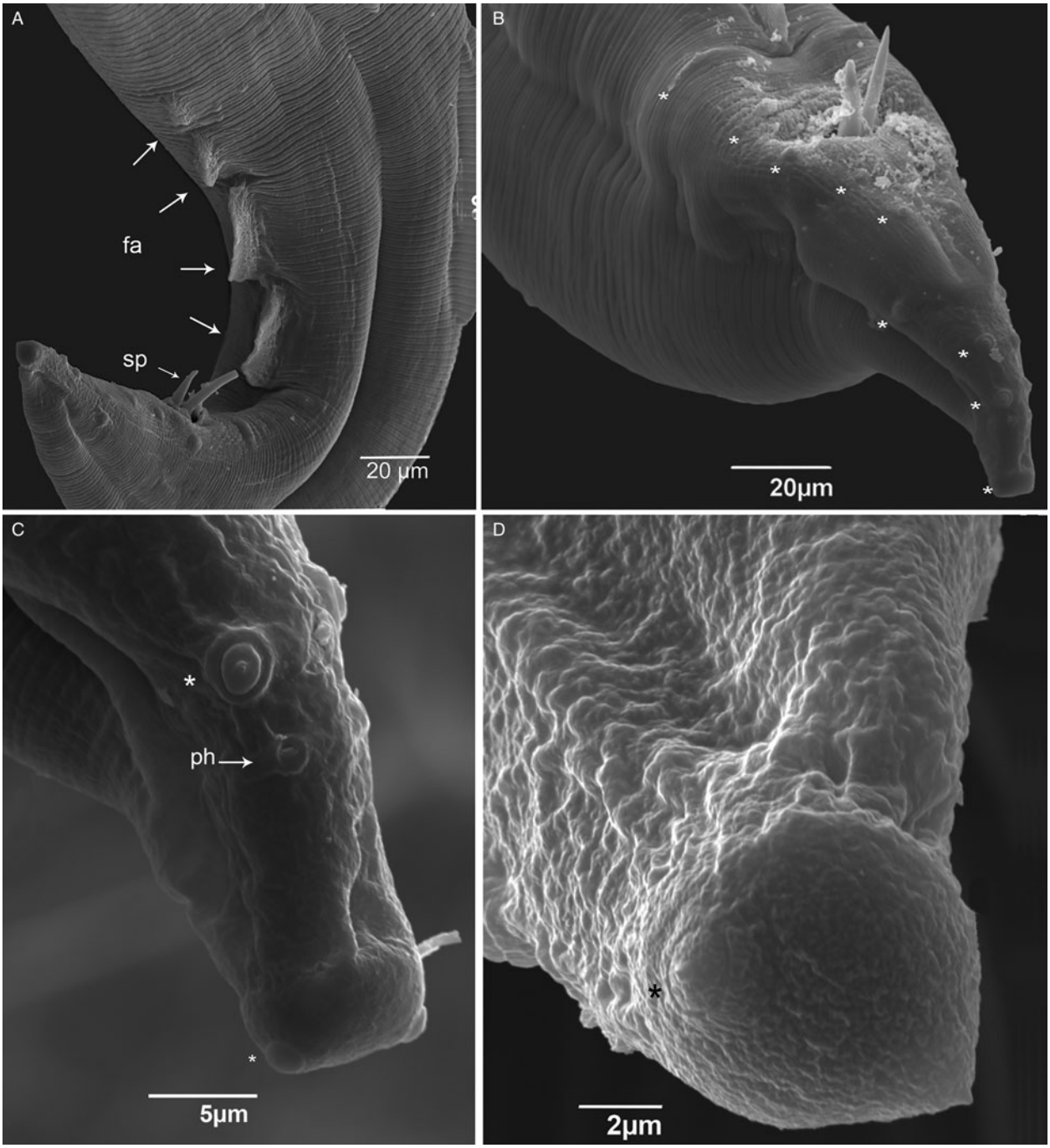

Fig. 3. Scanning electron microscopy of *P. (P.) zygodontomis*. (A) Male, posterior portion, ventral view, details of four fans (fa) and spicules (sp); (B) pairs of papillae (asterisk); (C) the tail, a pair of phasmids (ph) and papillae (asterisk); (D) tip of male with papillae (asterisk).

Sequences representing the superfamily Spiruroidea were not recovered as monophyletic in any topology. Within Spiruroidea, families Spiruridae, represented by *Protospirura* species, and Spirocercidae, represented by *Cylicospirura*, *Physocephalus* and *Spirocerca* species, were not monophyletic, although *Cylicospirura* and *Spirocerca* species formed a well-supported monophyletic group (LR-ELW = 99%, ML-BP = 97%, BPP = 100%).

Although relationships between superfamilies Rictularioidea, Filarioidea, Habronematoidea Physalopteroidea, Spiruroidea and Thelazioidea were generally poorly supported, their sequences formed a strongly supported monophyletic group (LR-ELW = 100%, ML-BP = 92%, BPP = 100%) (see supplementary table S1).

ML-pairwise genetic distances of the MT-CO1 gene between *Pterygodermatites* species ranged from 3 to 4%, between *P.* (*P.*) *jaergerskioldi* and *P.* (*P.*) *zygodontomis*. The intraspecific distances between *P.* (*P.*) *zygodontomis* specimens ranged from 0.04%, between the specimens from *N. lasiurus* and *Rhipidomys mastacalis* from Uberlândia, state of Minas Gerais, to 0.05% between the specimens from *N. lasiurus* from Uberlândia, state of Minas Gerais, and from Exú, state of Pernambuco. The intraspecific distance between *P.* (*P.*) *jaegerskioldi* specimens was 0.04% (table 3).

https://doi.org/10.1017/S0022149X17000736 Published online by Cambridge University Press

Table 2. Comparison of morphometric characteristics (in μm) of *Pterygodermatites (Paucepectines) zygodontomis* among specimens of *Necromys lasiurus* of Brazilian rodents.

Species	*P. zygodontomis*		*P. zygodontomis*		*P. zygodontomis*	
Reference	Quentin (1967)		Grossmann (2015)		Present study	
Distribution	Exú, Pernambuco, Brazil		Planaltina, Brasília, Brazil		Uberlândia, Minas Gerais, Brazil	
Sex (*n*)	Males	Females	Males (10)	Females (9)	Males (9)	Females (10)
Number cuticular formations (spine)	41	81/38 prevulvar	40–41	–	41	79–81/38–41 prevulvar
Total length (mm)	4.2 mm	28 mm	2.82 mm	12.96 mm	(2.85) 2.29–3.20 mm	(14) 10–19 mm
Body width	190	310	183	250	120–183	137.61–250.01
Length of buccal capsule	26	75	–	–	17.76–27	19.73–45.80
Base of buccal capsule	–	–	–	–	22.76–33	36.22–61.94
Total length of oesophagus	1200	3790	790	2550	768–994	1599–3290
Excretory pore (dfae)	250	1360	–	1000	186–259	379.82–522.41
Nerve ring (dfae)	400	350	140	180	135–184	152.39–278.46
Distance from the vulva to the oesophago-intestinal region	–	730	–	570	–	294.43–694
Vulva	–	4520	–	3110	–	1772–3950
Number of fans	3	–	–	–	3–4	–
Right spicule	55	–	54	–	45.12–61	–
Right spicule width	5	–	6	–	3.48–4.50	–
Left spicule	104	–	93	–	102.12–116	–
Left spicule width	10	–	8.1	–	9–10	–
Length of gubernaculum	42	–	–	–	30.6–43.78	–
Tail length	43	–	151	380	96.18–121	149.27–214
Total length spine anterior portion	50	–	40	–	35–55	33–57
Total length spine media portion	50	–	43	–	50–64	68–88
Total length spine posterior portion	60	–	50	–	44–58	46–75
Last spine-tail tip distance	–	770	–	–	–	226–498
Eggs (length)	–	37	–	–	–	31–50
Eggs (width)	–	28	–	–	–	16–26

dfae, Distance from anterior end.

Discussion

The genus *Pterygodermatites* is characterized by the oral opening shape, presence of three oesophageal teeth, and number of spine-like prevulvar cuticular projections. Moreover, the subgenus *Paucipectines* includes the arrangement of the caudal papillae in males (Quentin, 1969; Anderson *et al.*, 2009). In the Americas, 17 species belonging to the subgenus *Paucipectines* have been reported parasitizing rodents of the families Cricetidae and Sciuridae, marsupials of the families Caenolestidae and Didelphidae, and bats of the family Molossidae as definitive hosts (Lent & Freitas, 1935; Quentin, 1967; Sutton, 1979; Chabaud & Bain, 1981; Sutton, 1984; Navone, 1989; Navone & Suriano, 1992; Ramallo & Claps, 2007; Torres *et al.*, 2007; Jiménez & Patterson, 2012; Miño *et al.*, 2012; Lynggaard *et al.*, 2014).

Pterygodermatites (*Paucipectines*) *zygodontomis* was described as *Rictularia zygodontomis* by Quentin (1967) and later transferred to *Pterygodermatites* by Quentin (1969). Within the subgenus *Paucipectines*, this species can be distinguished from other species (*P. coloradensis*, *P. peromysci*, *P. parkeri*, *P. ondatrae*, *P. azarai*, *P. microti*, *P. onychomis* and *P. hymanae*) by the greater number of spine-like prevulvar cuticular projections (Lichtenfels, 1970; Lynggaard *et al.*, 2014). Furthermore, *P. kozeki*, *P. chaetophracti*, *P. spinicaudatis*, *P. microti* and *P. dipodomis* differ from *P. zygodontomis* due to the lower number of total spine-like cuticular projections in females.

In addition, *P. zygodontomis* differs from *P. baiomydis*, *P. azarai* and *P. elegans* by the smaller distance between the vulva and the oesophageal–intestinal junction. The nematodes *P. jaegerskioldi* and *P. massoiai*, both described only on females, are distinct from *P. zygodontomis* by the smaller distance from the spine-like cuticular projection to the tip of the tail (Sutton, 1979; Torres *et al.*, 2007).

https://doi.org/10.1017/S0022149X17000736 Published online by Cambridge University Press

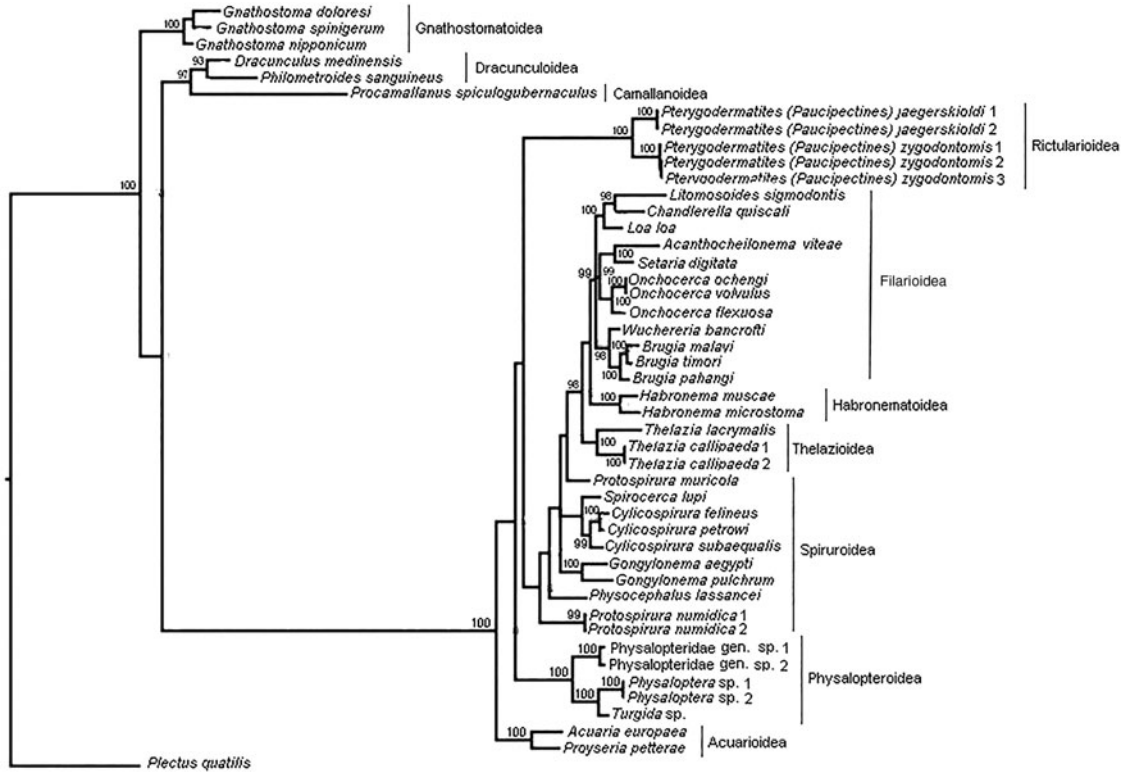

Fig. 4. Bayesian phylogenetic reconstruction, based on the MT-CO1 gene. Values at the nodes are the Bayesian posterior probabilities greater than 90%.

In the original description, *P. zygodontomis* was reported to present three unpaired fans in males in the posterior ventral region (Quentin, 1967). We observed four fans at the posterior ventral end using scanning electron microscopy. Probably, the first structure is not totally developed and cannot be visualized under light microscopy.

Although Quentin (1967) described males with ten pairs of papillae, we clearly observed a pair of phasmids between the eighth and ninth pairs of papillae at the posterior end. Thus, *P.* (*P.*) *zygodontomis* presented nine pairs of papillae and not ten as reported previously. To date, *P.* (*P.*) *zygodontomis* has been described as a parasite of *N. lasiurus*, sharing morphological characteristics such as the number of spine-like prevulvar cuticular projections and fans, in males, and unequal spicules. However, female specimens examined in this study and by Grossmann (2015) were smaller in body length than those found by Quentin (1967). Among the characteristics used to identify the species, we noted that the distance from the last spine-like cuticular projection to the tip of the tail in females was smaller than the distance observed by Quentin (1967), and the distance from the vulva to the oesophago-intestinal region was also smaller in the present study. In addition, we found differences in the distance from the cloaca to the tip of the tail in males. These differences may be due to different development stages of the specimens analysed by Quentin and in the present study.

Table 3. Interspecific and intraspecific MT-CO1 pairwise ML genetic distances, minimum and maximum percentages within spirurid genera.

	Interspecific	Intraspecific
Brugia	0.76–1.45	*
Cylicospirura	0.57–1.67	*
Gnathostoma	2.10–2.92	*
Habronema	2.30	*
Onchocerca	0.05–1.72	*
Physaloptera	*	0.003
Physalopteridae gen.	0.51	*
Protospirura	4.95–4.98	0.103
Pterygodermatites	3.25–4.09	0.035–0.053
Thelazia	5.23–5.30	0.016

*, Not applicable.

The original morphological description of *P.* (*P.*) *zygodontomis* by Quentin (1967) was confirmed in the present study using light microscopy and SEM. We also added new characteristics using SEM, such as details of the oral opening surrounded by a toothed strip of 21 denticles, four fans and a pair of phasmids.

Additionally, our findings increase the host spectrum and the geographic distribution of *P.* (*P.*) *zygodontomis*, through the description of this species infecting *N. lasiurus* and *R. mastacalis* in the Cerrado biome, while the original description of the species was restricted to *N. lasiurus* individuals in the Caatinga biome. Cerrado and Caatinga are neighbouring biomes forming the so-called South American Dry Diagonal, a large belt of land characterized by high seasonality and low rainfall. Their mammal faunas are largely shared (120 species, including 22 rodents) (Carmignotto *et al.*, 2012), which contributes to similarities in the helminth fauna of mammalian hosts. The presence of this parasite in both biomes may be linked to the extended distribution of *N. lasiurus*. Moreover, infection of *R. mastacalis* by *P.*

https://doi.org/10.1017/S0022149X17000736 Published online by Cambridge University Press

(*P.*) *zygodontomis* may be accidental, although indicating the low host-specificity of this parasite. Distribution and feeding habits in these rodents may explain their infection by *P.* (*P.*) *zygodontomis*. Rodents of both species (*N. lasiurus* and *R. mastacalis*) inhabit the Cerrado biome (Carmignotto *et al.*, 2012) and have an insectivorous–omnivorous diet (Talamoni *et al.*, 2008; Pinotti *et al.*, 2011), which may favour infection through the ingestion of insects that act as intermediate hosts. However, while *Necromys* is a genus endemic to open formations (D'Elia, 2003), *R. mastacalis* is an arboreal species, commonly found in forest physiognomies (Atlantic Forest, woodlands and gallery or semi-deciduous forests in the Cerrado biome), although also occurring in the Caatinga. Hence, conversion of natural ecosystems to open formations, following anthropogenic disturbances occurring in the Cerrado during the past few decades, may have contributed to the transmission dynamics of *P.* (*P.*) *zygodontomis* between rodent species.

In our molecular phylogenies, based on the MT-CO1 gene, no phylogenetic affinities could be unambiguously established between *Pterygodermatites* species and other Spirurid families or superfamilies. In addition, a close relationship between families Rictulariidae and Physalopteridae, as suggested by Vicente *et al.* (1997), was not supported in our phylogenetic analysis. Nevertheless, we have found strong support for a group including the superfamilies Filarioidea, Habronematoidea Physalopteroidea, Rictularioidea, Spiruroidea and Thelazioidea. This group is consistent with the classifications proposed by Ley & Blaxter (2002), for an infraorder Spiruromorpha, or by Hodda (2011), for a suborder Spirurina, once excluding Camallanoidea; or is consistent with the classification proposed by Anderson *et al.* (2009), for a suborder Spirurina, once excluding Gnathostomatoidea. Conversely, Gnathostomatoidea, Dracunculoidea and Camallanoidea were more distantly related to other spirurids; with a Dracunculoidea–Camallanoidea clade supporting the suborder Camallanina proposed by Anderson *et al.* (2009).

Additionally, in topologies, our sequences of *P. numidica* did not form a clade with the sequence of *Protospirura muricola* from GenBank. That would not be expected if these specimens belonged to the same genus. Presumably, the sample from GenBank may have been erroneously assigned to the genus *Prostospirura*, while in fact belonging to the genus *Mastophorus*. Because of the inappropriate choice of characters, *Protospirura* has had a long and difficult history of confusion with the similar *Mastophorus*, as stated by Smales *et al.* (2009). A complete morphological examination of the GenBank specimen of *P. muricola* could clear this matter. Moreover, genetic distances between *Protospirura* species were approximately 5%, which were larger than the distances between species of *Brugia*, *Cylicospirura*, *Gnathostoma*, *Habronema*, *Onchocerca* and *Pterygodermatites*, and smaller than the distances between *Thelazia* species.

In conclusion, the geographic distribution of *P.* (*P*) *zygodontomis* was expanded with this study and new morphological characters were added. In addition, this is the first report of molecular phylogenetic analyses of the Rictulariidae family, enhancing its molecular dataset and contributing to future research that may compare ancestry among the *Pterygodermatites* subgenera, as proposed by Quentin (1969). Further studies are needed for a better understanding of the relationships between the spirurid superfamilies, including representatives of the Aproctoidea and Diplotriaenoidea superfamilies.

Supplementary material. To view supplementary material for this article, please visit https://doi.org/10.1017/S0022149X17000736

Acknowledgements. We thank Dr Paulo D'Andrea for providing us with his ICMBio licence; Dr Helene Santos Barbosa for the use of the Rudolf Barth Electron Microscopy Platform (PDTIS/FIOCRUZ); and Socrates Costa-Neto from the Laboratório de Biologia e Parasitologia de Mamíferos Silvestres Reservatórios for assistance with the collection of rodents and helminths. We also thank the staff of the Plataforma de Sequenciamento de DNA do Instituto Oswaldo Cruz (PDTIS/FIOCRUZ) for assistance with sequencing samples in this study. Finally, we are grateful to Dr Marcelo Knoff for loaning specimens from the Coleção Helmintológica do Instituto Oswaldo Cruz (CHIOC).

Financial support. This study received financial support from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior-CAPES, Instituto Oswaldo Cruz (FIOCRUZ), CNPq and FAPEMIG.

Conflict of interest. None.

Ethical standards. Licences for animal capture were provided by Chico Mendes Institute for Biodiversity Conservation (ICMBio authorization number 13373). All protocols followed the guidelines for capture, handling and care of the Ethics Committee on Animal Use of the Oswaldo Cruz Institute (according to licences L-049/08 and 066/08).

References

Anderson RC, Chabaud AG and Willmott S (2009) *Keys to the nematode parasites of vertebrates*. 463 pp. Wallingford, CABI.

Blaxter ML, De Ley P, Garey JR, Liu LX, Scheldeman P, Vierstraete A and Vida JT (1998) A molecular evolutionary framework for the phylum Nematoda. *Nature* **392**, 71–75.

Bonvicino C.R, Oliveira JA and D'Andrea PS (2008) *Guia dos Roedores do Brasil, com chavez para gêneros baseadas em caracteres externos*. 120pp. Rio de Janeiro, Centro Pan-Americano de Febre Aftosa – OPAS/OMS.

Brandão-Filho SP, Brito M.E, Carvalho FG, Ishikawa EA, Cupolillo E, Floeter-Winter L and Shaw JJ (2003) Wild and synantrophic hosts of *Leshmania* (*Viannia*) *braziliensis* in the endemic cutaneous leishmaniasis locality of Amaraji, Pernambuco State, Brazil. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **37**, 291–296.

Cardoso TS, Simões RO, Luque JLF, Maldonado A and Gentile R (2016) The influence of habitat fragmentation on helminth communities in rodent populations from a Brazilian Mountain Atlantic Forest. *Journal of Helminthology* **90**, 460–468.

Carmignotto AP, Vivo M de and Langguth A (2012) Mammals of the Cerrado and Caatinga: distribution patterns of the tropical open biomes of Central South America. pp. 307–350 *in* Patterson BD and Costa LP (*Eds*) *Bones, clones, and biomes: the history and geography of recent Neotropical mammals*. Chicago, University of Chicago Press.

Casiraghi M, Anderson TJ, Bandi C, Bazzocchi C and Genchi C (2001) A phylogenetic analysis of filarial nematodes: comparison with the phylogeny of *Wolbachia* endosymbionts. *Parasitology* **122**, 93–103.

Chabaud AG and Bain O (1981) *Quentius kozeki* n. g., n. sp., Nématode rictulaire parasite d'um Marsupial américain. *Annales de Parasitologie* **56**, 175–178.

Chaudhary A, Verma C, Tomar V and Singh HS (2017) *Procamallanus spiculogubernaculus* Agarwal, 1958 (Nematoda: Camallanidae) from Stinging catfish, *Heteropneustes fossilis* in India: morphological characterization and molecular data. *Helminthologia* **54**, 1–9.

Crainey JL, da Silva TRR, Encinas F, Marín MA, Vicente ACP and Luz SLB (2016) The mitogenome of *Onchocerca volvulus* from the Brazilian Amazonia focus. *Memórias do Instituto Oswaldo Cruz* **111**, 79–81.

D'Elia G (2003) Phylogenetics of Sigmodontinae (Rodentia, Muroidea, Cricetidae), with special reference to the Akodont group and with additional comments on historical biogeography. *Cladistics* **19**, 307–323.

Ferguson J.A, Woodberry K, Gillin CM, Jackson DH, Sanders JL, Madigan W, Bildfell RJ and Kent ML (2011) *Cylicospirura* species (Nematoda: Spirocercidae) and stomach nodules in cougars (*Puma concolor*) and bobcats (*Lynx rufus*) in Oregon. *Journal of Wildlife Diseases* **47**, 140–153.

Francisco AL, Magnusson WE and Sanaiotti TM (1995) Variation growth and reproduction of *Bolomys lasiurus* (Rodentia: Muridae) in an Amazonian Savanna. *Journal of Tropical Ecology* **11**, 419–428.

Gardner SL and Campbell MI (1992) Parasites as probes for biodiversity. *Journal of Parasitology* **78**, 596–600.

Gentile R, Costa Neto SF and D'Andrea PS (2010) Uma revisão sobre a participação do rato d'água *Nectomys squamipes* na dinâmica de transmissão da esquistossomose mansônica: Um estudo multidisciplinar de longo prazo em uma área endêmica. *Oecologia Australis* **14**, 711–725.

Ghedin E, Wang S, Spiro D, *et al.* (2007) Draft genome of the filarial nematode parasite *Brugia malayi*. *Science* **80**, 1756–1760.

Grenfell BT (1992) Parasitism and the dynamics of ungulate grazing systems. *American Naturalist* **139**, 907–929.

Grossmann NV (2015) *Relações Parasito-Hospedeiro de Endo e Ectoparasitas em Pequenos Mamíferos em um Cerrado do Brasil Central*. 167 pp. Brasília, Universidade de Brasília, Instituto de Ciências Biológicas.

Hodda M (2011) Phylum Nematoda Cobb 1932. pp. 63–95 *in* Zhang Z.-Q. (*Ed.*) *Animal biodiversity: An outline of higher-level classification and survey of taxonomic richness*. Auckland, NZ, Magnolia Press.

Iorio R, Šlapeta J, Otranto D, Paoletti B, Giangaspero A and Traversa D (2009) Phylogenetic relationships of *Habronema microstoma* and *Habronema muscae* (Spirurida: Habronematidae) within the order Spirurida inferred using mitochondrial cytochrome c oxidase subunit 1 (cox1) gene analysis. *Parasitological Research* **104**, 979–984.

Jiménez FA and Patterson BD (2012) A new species of *Pterygodermatites* (Nematoda: Rictulariidae) from the Incan shrew opossum, *Lestoros inca*. *Journal of Parasitology* **98**, 604–607.

Jobb G. (2011) TREEFINDER version of March 2011. Munich. Distributed by the author at www.treefinder.de (accessed 16 August 2015).

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Mentjies P and Drummond A (2012) Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* **28**, 1647–1649.

Kim J, Kern E, Kim T, Sim M, Kim J, Kim Y, Park C, Nadler SA and Park JK (2017) Phylogenetic analysis of two *Plectus* mitochondrial genomes (Nematoda: Plectida) supports a sister group relationship between Plectida and Rhabditida within Chromadorea. *Molecular Phylogenetics and Evolution* **107**, 90–102.

Lefoulon E, Bain O, Bourret J, Junker K, Guerrero R, Cañizales I, Kuzmin Y, Satoto TBT, Cardenas-Callirgos JM, de Souza Lima S, Raccurt C, Mutafchiev Y, Gavotte L and Martin C (2015) Shaking the tree: multi-locus sequence typing usurps current Onchocercid (filarial nematode) phylogeny. *PLoS Neglected Tropical Diseases* **9**, 1–19.

Lemos ERS and D'Andrea PS (2014) *Trabalho com animais silvestres: procedimentos, riscos e biossegurança*. 180 pp. Rio de Janeiro, Editora Fiocruz.

Lent H and Freitas JF (1935) Sobre dois novos nematódeos parasitos de quica: *Caluromys philander*. *Memórias do Instituto Oswaldo Cruz* **30**, 535–542.

Ley P and Blaxter ML (2002) Systematic position and phylogeny. pp. 1–30 *in* Lee D (*Ed.*) *The biology of nematodes*. London, Taylor & Francis.

Lichtenfels JR (1970) Two new species of *Pterygodermatites* (*Paucipectines*) Quentin, 1969 (Nematoda: Rictulariidae) with a key to the species from North American rodents. *Proceedings of the Helminthological Society of Washington* **37**, 94–101.

Limongi JE, Moreira FG, Peres JB, Suzuki A, Ferreira IB, Souza RP, Pinto RMC and Pereira LE (2013) Serological survey of hantavirus in rodents in Uberlândia, Minas Gerais, Brazil. *Revista Instituto de Medicina Tropical* **55**, 155–158.

Liu GH, Gasser RB, Otranto D, Xu MJ, Shen JL, Mohandas N, Zhou DH and Zhu XQ (2013a) Mitochondrial genome of the eyeworm, *Thelazia callipaeda* (Nematoda: Spirurida), as the first representative from the family Thelaziidae. *PLoS Neglected Tropical Diseases* **7**, e2029.

Liu GH, Wang Y, Song HQ, Li MW, Ai L, Yu XL and Zhu XQ (2013b) Characterization of the complete mitochondrial genome of *Spirocerca lupi*: sequence, gene organization and phylogenetic implications. *Parasites & Vectors* **6**, 45–54.

Liu GH, Jia YQ, Wang YN, Zhao GH and Zhu XQ (2015a) The complete mitochondrial genome of the gullet worm *Gongylonema pulchrum*: gene content, arrangement, composition and phylogenetic implications. *Parasites & Vectors* **8**, 1–8.

Liu GH, Shao R, Cai XQ, Li WW and Zhu XQ (2015b) *Gnathostoma spinigerum* mitochondrial genome sequence: a novel gene arrangement and its phylogenetic position within the class Chromadorea. *Scientific Reports* **5**, 12691.

Liu GH, Li JY and Zhu XQ (2017) Characterization of the complete mitochondrial genome of *Setaria digitata* (Nematoda: Setariidae) from China. *Journal of Helminthology*, 1–5. doi:10.1017/S0022149X16000912.

Lynggaard C, García-Prieto L, Guzmán-Cornejo C and Osorio-Sarabia D (2014) *Pterygodermatites* (*Paucipectines*) *baiomydis* n. sp. (Nematoda: Rictulariidae), a parasite of *Baiomys taylori* (Cricetidae). *Parasite* **21**, 58.

Mafra AC and Lanfredi RM (1998) Reevaluation of *Physaloptera bispiculata* (Nematoda: Spiruroidaea) by light and scanning electron microscopy. *Journal of Parasitology* **84**, 582–588.

McNulty SN, Mullin AS, Vaughan JA, Tkach V.V, Weil GJ and Fischer PU (2012) Comparing the mitochondrial genomes of *Wolbachia*-dependent and independent filarial nematode species. *BMC Genomics* **13**, 145–155.

Miño MH, Rojas Herrera EJ, Notarnicola J, Robles MR and Navone GT (2012) Diversity of the helminth community of the Pampean grassland mouse (*Akodon azarae*) on poultry farms in central Argentina. *Journal of Helminthology* **86**, 46–53.

Morand S and Arias-Gonzalez E (1997) Is parasitism a missing ingredient in model ecosystems? *Ecological Modeling* **95**, 61–74.

Morand S, Ivanova ES and Vaucher C (1996) *Dicelis keymeri* n. sp. (Nematoda: Drilonematidae) from the earthworm *Octalasium pseudotranspadanum* Zicsi. *Proceedings of the Helminthological Society of Washington* **63**, 19–23.

Moreira VLC, Giese EG, Melo FTV, Simões RO, Thiengo SC, Maldonado A Jr and Santos JN (2013) Endemic angiostrongyliasis in the Brazilian Amazon: natural parasitism of *Angiostrongylus cantonensis* in *Rattus rattus* and *R. norvegicus* and sympatric giant African land snails, *Achatina fulica*. *Acta Tropica* **125**, 90–97.

Mutafchiev Y, Mariaux J and Georgiev BB (2014) Description of *Proyseria petterae* n. sp., with an amended generic diagnosis and a review of the species of *Proyseria* Petter, 1959 and *Stegophorus* Wehr, 1934 (Nematoda: Acuariidae). *Systematic Parasitology* **89**, 3–14.

Mutafchiev Y, Mariaux J and Georgiev BB (2017) Description of *Acuaria europaea* n. sp. (Spirurida: Acuariidae) from *Dendrocopos syriacus* (Hemprich & Ehrenberg) and *Oriolus oriolus* (L.) (Aves) in Europe, with results of re-examination of related European species of *Acuaria* Bremser, 1811. *Systematic Parasitology* **94**, 201–214.

Nadler SA, Carreno RA, Mejía-Madrid H, Ullberg J, Pagan C, Houston R and Hugot JP (2007) Molecular phylogeny of clade III nematodes reveals multiple origins of tissue parasitism. *Parasitology* **134**, 1421–1442.

Navone GT (1989) *Pterygodermatites* (*Paucipectines*) *kozeki* (Chabaud et Bain, 1981) n. comb., parásito de Lestodelphys halli Tate, 1934, *Didelphis albiventris* L. y *Thylamis pusilla* (Desmarest) de la República Argentina. Anatomía y posición sistemática. *Revista Ibérica de Parasitología* **49**, 219–226.

Navone GT and Suriano DM (1992) *Pterigodermatites* (*Paucipectines*) *spinicaudatis* n. sp. (Nematoda: Rictulariidae) from *Dromiciops australis* (Marsupialia: Microbiotheriidae) in Bariloche, Rio Negro, Argentina. Biogeographical distribution and host–parasite relationships. *Memórias do Instituto Oswaldo Cruz* **87**, 533–538.

Oliveira RC, Guterres A, Fernandes J, D'Andrea PS, Bonvicino CR and de Lemos ERS (2014) Hantavirus reservoirs: current status with an emphasis on data from Brazil. *Viruses* **6**, 1929–1973.

Orozco MM, Piccinali RV, Mora MS, Enriquez GF, Cardinal MV and Gürtler RE (2014) The role of sigmodontine rodents as sylvatic hosts of *Trypanosoma cruzi* in the Argentinean Chaco. *Infection Genetics and Evolution* **22**, 12–22.

Pinotti BT, Naxara L and Pardini R (2011) Diet and food selection by small mammals in an old-growth Atlantic forest of south-eastern Brazil. *Studies on Neotropical Fauna and Environment* **46**, 1–9.

Poulin R (1998) Comparison of three estimators of species richness in parasite component communities. *Journal of Parasitology* **84**, 485–490.

Prosser SWJ, Velarde-Aguilar MG, León-Règagnon V and Hebert PDN (2013) Advancing nematode barcoding: a primer cocktail for the

cytochrome c oxidase subunit I gene from vertebrate parasitic nematodes. *Molecular Ecology Resources* **13**, 1108–1115.

Quentin JC (1967) *Rictularia zygodontomis* n. sp., nématode nouveau parasite de rongeurs Du Brésil. *Bulletin du Muséum National D'Histoire Naturelle, Paris* **39**, 740–744.

Quentin JC (1969) Essai de classification des nématodes rictulaires. *Mémoires du Muséum National D'histoire Naturalle* **LIV**, 1–115.

Ramallo G and Claps LE (2007) Nuevos hospedadores y registros geograficos de *Pterygodermatites* (*Paucipectines*) *kozeki* (Nematoda, Rictulariidae) en Argentina. *Mastozoología Neotropical* **14**, 93–96.

Rambaut A (2012) FigTree, a graphical viewer of phylogenetic trees. Available at http://tree.bio.ed.ac.uk/software/figtree (accessed 5 May 2017).

Ramesh A, Small ST, Kloos ZA, Kazura JW, Nutman TB, Serre D and Zimmerman PA (2012) The complete mitochondrial genome sequence of the filarial nematode *Wuchereria bancrofti* from three geographic isolates provides evidence of complex demographic history. *Molecular and Biochemical Parasitology* **183**, 32–41.

Redford K and Eisenberg J (1999) *Mammals of the Neotropics*. 3rd edn. 624 pp. Chicago, The University of Chicago Press.

Ronquist F, Teslenko M, van derMark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA and Huelsenbeck JP (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* **61**, 539–542

Sabino-Santos G, Maia FGM, Jonsson CB, Goodin DG, Salazar-Bravo J and Figueiredo LTM (2016) Serologic evidence of mammarenaviruses among wild rodents in Brazil. *Journal of Wildlife Diseases* **52**, 766–769.

Schwarz G (1978) Estimating the dimension of a model. Institute of Mathematical Statistics. *Annals of Statistics* **6**, 461–464.

Setsuda A, Da N, Hasegawa H, Behnke JM, Rana HB, Dhakal IP and Sato H (2016) Intraspecific and interspecific genetic variation of *Gongylonema pulchrum* and two rodent *Gongylonema* spp. (*G. aegypti* and *G. neoplasticum*), with the proposal of *G. nepalensis* n. sp. for the isolate in water buffaloes from Nepal. *Parasitological Research* **115**, 787–795.

Simões R, Gentile R, Rademaker V, D'Andrea P, Herrera H, Freitas T, Lanfredi R and Maldonado A (2010) Variation in the helminth community structure of *Thrichomys pachyurus* (Rodentia: Echimyidae) in two subregions of the Brazilian Pantanal: the effects of land use and seasonality. *Journal of Helminthology* **84**, 266–275.

Smales LR, Harris PD and Behnke JM (2009) A redescription of *Protospirura muricola* Gedoelst, 1916 (Nematoda: Spiruridae), a parasite of murid rodents. *Systematic Parasitology* **72**, 15–26.

Su YB, Kong SC, Wang LX, Chen L and Fang R (2016) Complete mitochondrial genome of *Philometra carassii* (Nematoda: Philometridae). *Mitochondrial DNA. Part A, DNA mapping, sequencing, and analysis* **27**, 1397–1398.

Sun MM, Ma J, Sugiyama H, Ando K, Li WW, Xu QM, Liu GH and Zhu XQ (2016) The complete mitochondrial genomes of *Gnathostoma doloresi* from China and Japan. *Parasitological Research* **115**, 4013–4020.

Sun MM, Liu GH, Ando K, Woo HC, Ma J, Sohn WM, Sugiyama H and Zhu XQ (2017) Complete mitochondrial genomes of *Gnathostoma nipponicum* and *Gnathostoma* sp., and their comparison with other *Gnathostoma* species. *Infection, Genetics and Evolution* **48**, 109–115.

Sutton CA (1979) Contribución al conocimiento de la fauna parasitológica Argentina. IV. *Rictularia massoiai* sp. n. y *Enterobius yagoi* sp. n. (Nematoda). *Acta Zoologica Lilloana* **35**, 29–37.

Sutton CA (1984) Contribución al conocimiento de la fauna parasitológica Argentina XIII Nuevos nematodes de la familia Rictulariidae. *Neotropica* **30**, 141–152.

Talamoni SA, Couto D, Cordeiro DA Júnior and Diniz FM (2008) Diet of some species of Neotropical small mammals. *Mammalian Biology* **73**, 337–341.

Thomas F, Renaud F and Guégan JF (2005) *Parasitism and ecosystems*. 229 pp. New York, Oxford University Press.

Torres EL, Maldonado A Jr and Lanfredi RM (2007) *Pterygodermatites* (*Paucipectines*) *jägerskiöldi* (Nematoda: Rictulariidae) from *Gracilinanus agilis* and *G. microtarsus* (Marsupialia: Didelphidae) in Brazilian Pantanal and Atlantic Forest by light and scanning electron microscopy. *Journal of Parasitology* **93**, 274–279.

Vicente JJ, Rodrigues HO, Gomes DC and Pinto RM (1997) Nematoides do Brasil. Parte V: Nematóides de Mamíferos. *Revista Brasileira de Zoologia* **14** (Suppl. 1), 1–452.

Vieira EM and Baumgarten LC (1995) Daily activity patterns of small mammals in a Cerrado Area from central Brazil. *Journal of Tropical Ecology* **11**, 255–262.

Wijová M, Moravec F, Horak A, Modry D and Lukes J (2005) Phylogenetic position of *Dracunculus medinensis* and some related nematodes inferred from 18S rRNA. *Parasitology Research* **96**, 133–135.

Xia X (2017) DAMBE6: New tools for microbial genomics, phylogenetics and molecular evolution. *Journal of Heredity* **108**, 431–437.

Xia X and Lemey P (2009) Assessing substitution saturation with DAMBE. pp. 615–630 *in* Lemey P, Salemi M and Vandamme AM (*Eds*) *The phylogenetic handbook: A practical approach to DNA and protein phylogeny*. 2nd edn. Cambridge, Cambridge University Press.

Xia X, Xie Z, Salemi M, Chen L and Wang Y (2003) An index of substitution saturation and its application. *Molecular Phylogenetics and Evolution* **26**, 1–7.